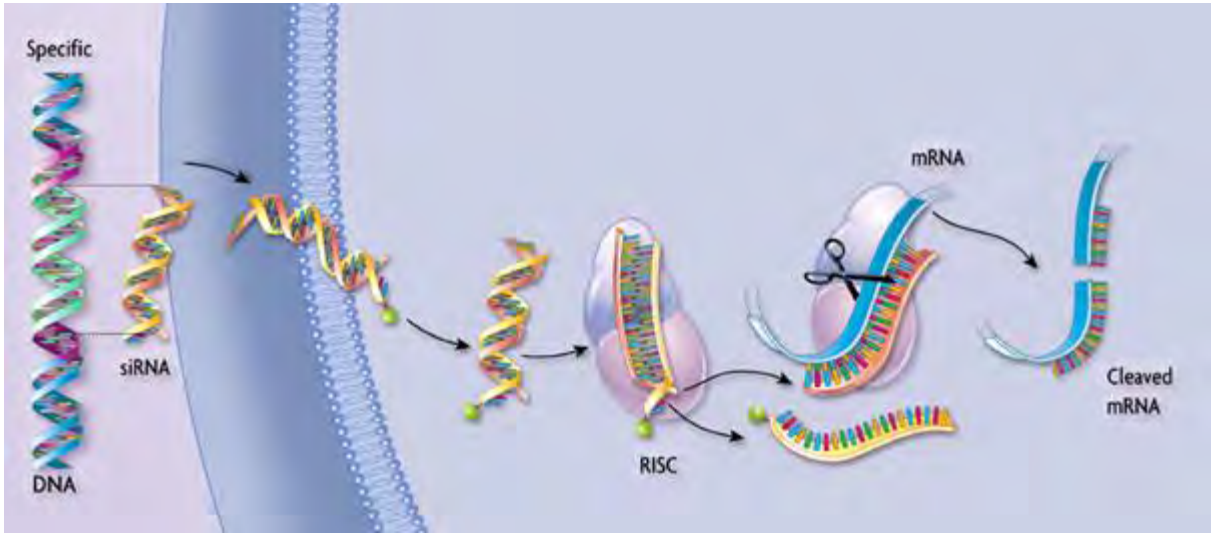
Small Interfering RNA's – Molecular biology and use in therapy



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Small Interfering RNA's

- Small Interfering RNA's (siRNA's) are a class of double-stranded RNA molecules 20-25 base pairs in length that operate within the RNA interferance pathway.

- They interfer with expression of specific genes with complementary nucleotide sequences by degrading mRNA and thereby inhibiting gene translation

- First characterised in 1999 they have now become a viable therapeutic option to treat a variety of pathologies including viral infection and virus-induced disease

Small Interfering RNA's

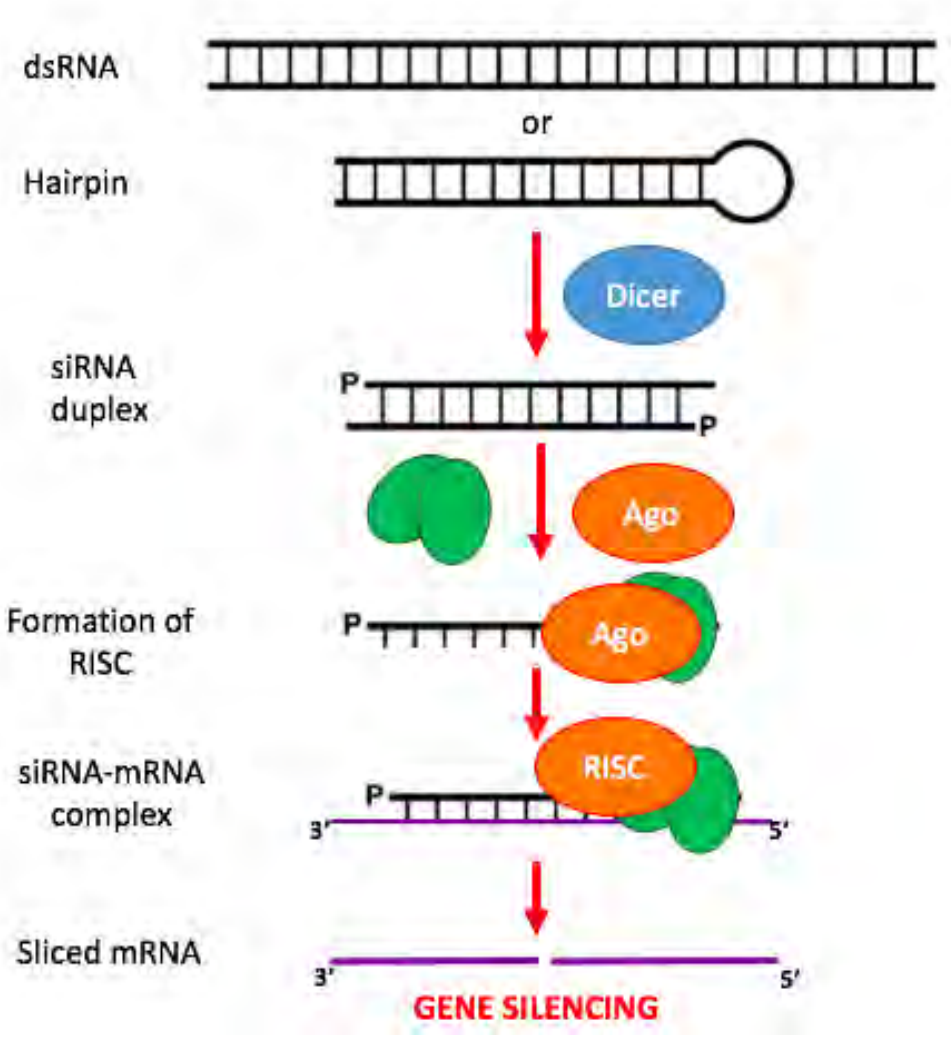
A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants

Andrew J. Hamilton and David C. Baulcombe*

Posttranscriptional gene silencing (PTGS) is a nucleotide sequence–specific defense mechanism that can target both cellular and viral mRNAs. Here, three types of transgene-induced PTGS and one example of virus-induced PTGS were analyzed in plants. In each case, antisense RNA complementary to the targeted mRNA was detected. These RNA molecules were of a uniform length, estimated at 25 nucleotides, and their accumulation required either transgene sense transcription or RNA virus replication. Thus, the 25-nucleotide antisense RNA is likely synthesized from an RNA template and may represent the specificity determinant of PTGS.

Science 29 Oct 1999:
Vol. 286, Issue 5441, pp. 950-952

siRNA Mechanism of Action



siRNA as Therapeutics

- Recent developments in siRNA technology:
  - reduced toxicity
  - reduced immunostimulatory effects
  - reduced off target effects
  - higher efficacies
  - increased stability
  - design of specific siRNA nanoparticle delivery vehicles

siRNA Therapeutics for Viral Infection

- Recent *in vitro* studies have demonstrated the effectiveness of siRNA inhibition of viral infection including

  - human cytomegalovirus
  - human immunodeficiency virus-1
  - hepatitis B virus
  - hepatitis C virus
  - poliovirus
  - human papillomavirus
  - influenza virus

Congenital Cytomegalovirus (CMV) Disease

- In developed countries, CMV is leading non-genetic cause of congenital infection and fetal malformation

- Results in development of serious clinical sequale including hearing loss, vision loss, mental disability or in severe cases fetal and neonatal death

- Infection of the placenta is a pre-requisite for infection of the fetus

- Fetal injury results from direct viral cytopathic damage to the CMV-infected fetus and from the indirect effects of placental infection alone

Therapeutics for Prevention and Treatment of Congenital CMV Infection during Pregnancy

- Common anti-viral CMV therapies (e.g. ganciclovir) are unsuitable for use during pregnancy due to toxicity and limited evidence for efficacy.

- Hyperimmune globulin
  - Passive immunisation using high avidity neutralising antibodies directed against CMV
  - Conflicting evidence on the effectiveness of treatment
  - Does not decrease viral load, low supply, expensive etc.

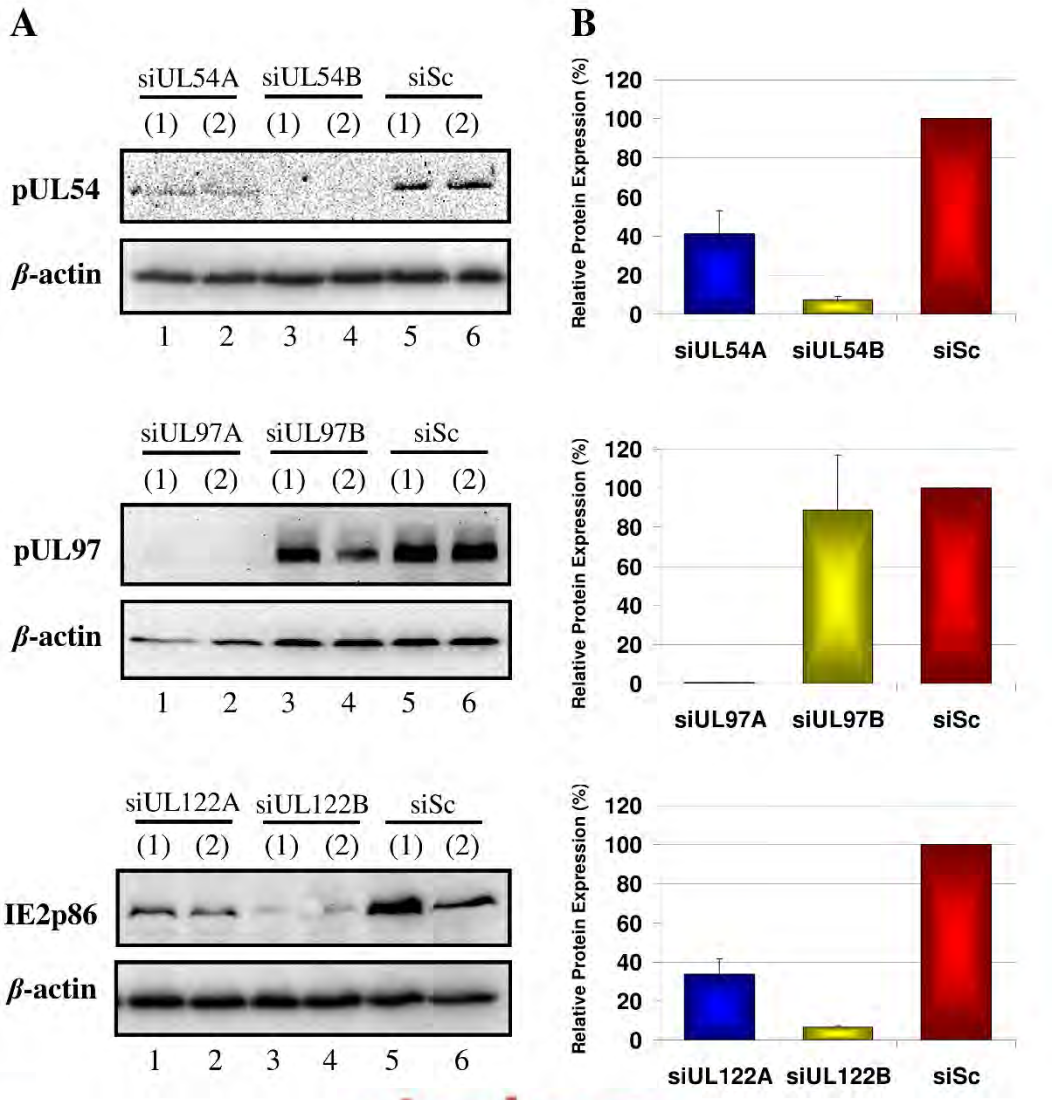
- siRNA as potential therapy
  - siRNA's can be developed to target essential genes for CMV replication

siRNA Inhibition of CMV Replication Targeting CMV Essential Genes

CMV UL122, UL54 and UL97 genes are essential for viral replication

- UL122 immediate early gene transcript
  - UL122 expression is essential for early and late CMV gene expression
- UL54 early gene transcript
  - Encodes for the viral DNA polymerase
- UL97 early gene transcript
  - Encodes for the viral protein kinase

siRNA Inhibition of CMV Protein Expression



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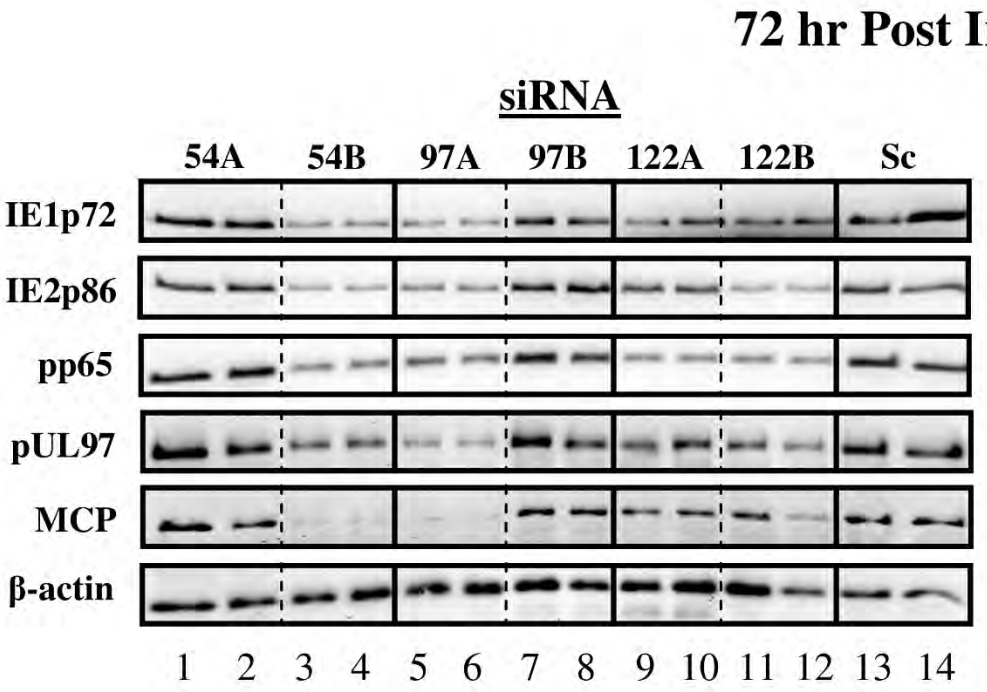
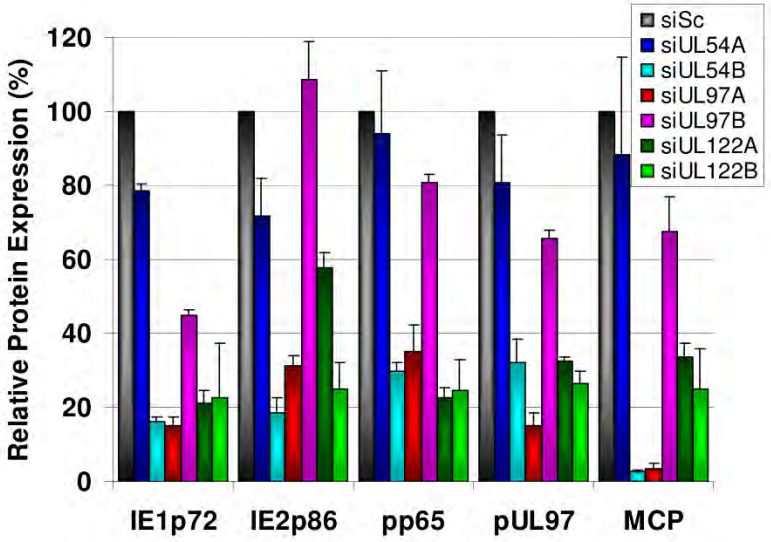
siRNA Inhibition of CMV Protein Expression After Single Round of Replication (1 pfu/cell) 72hpi





Hamilton *et al.* 2014 PLoS ONE

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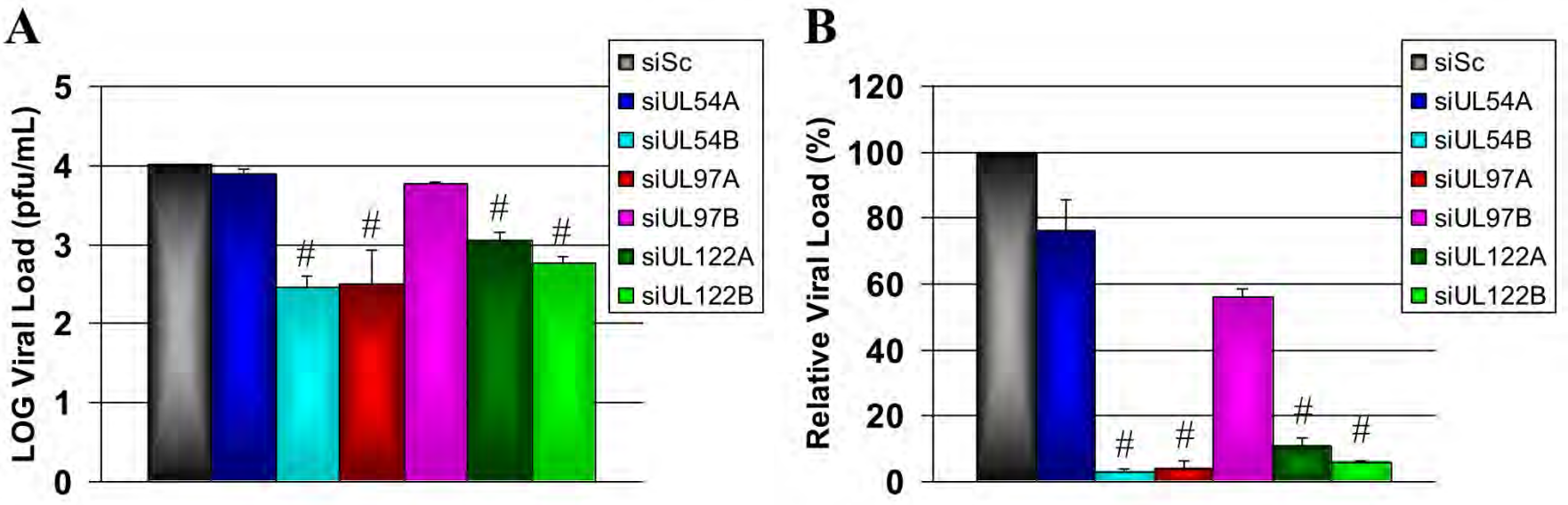
siRNA Inhibition of CMV Progeny Production After Single Round of Replication (1 pfu/cell) 72hpi



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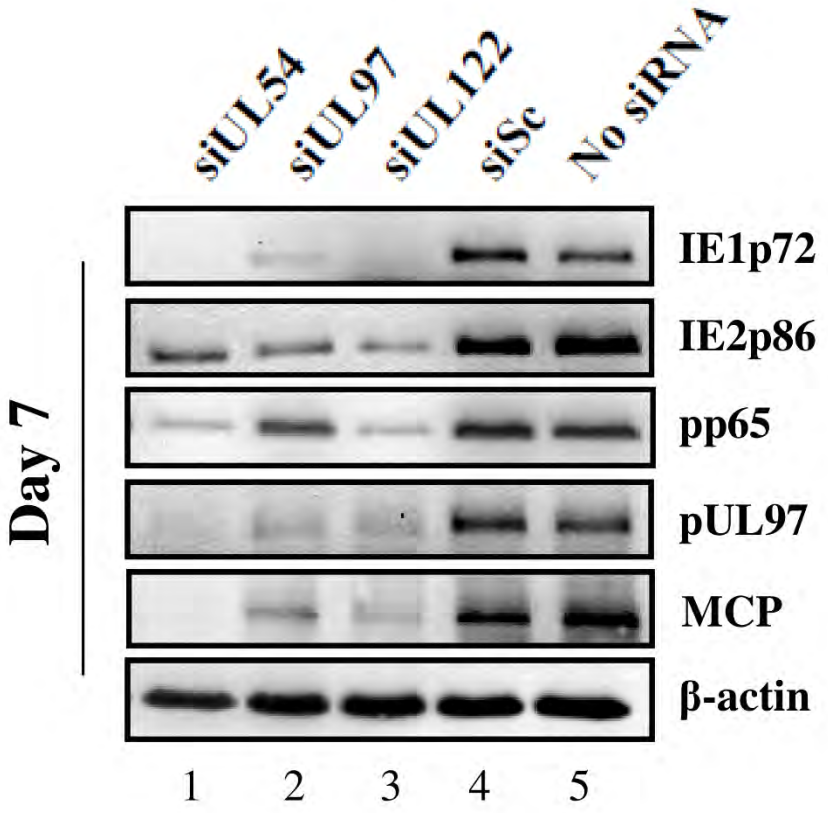
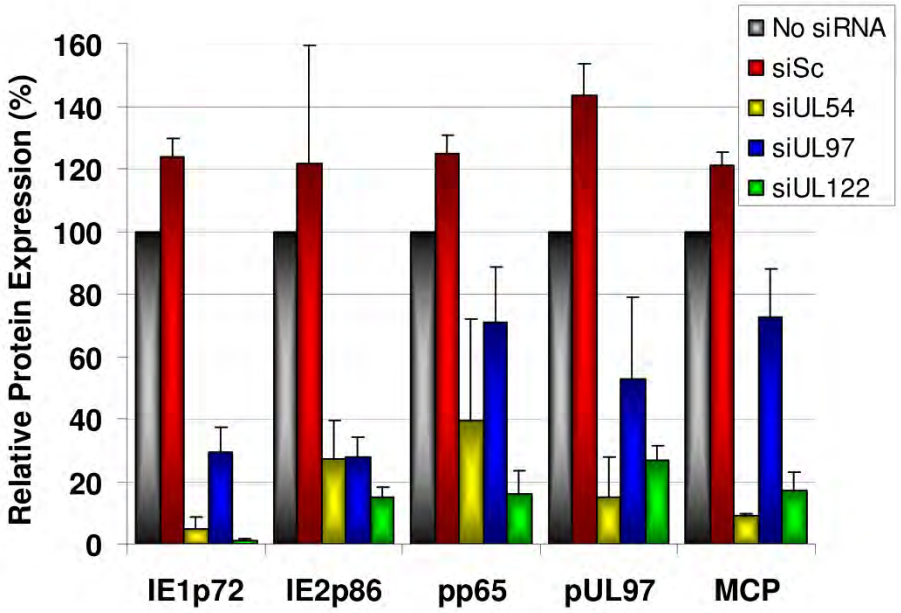
siRNA Inhibition of CMV Protein Expression After Multiple Rounds of Replication (0.001 pfu/cell) 7dpi





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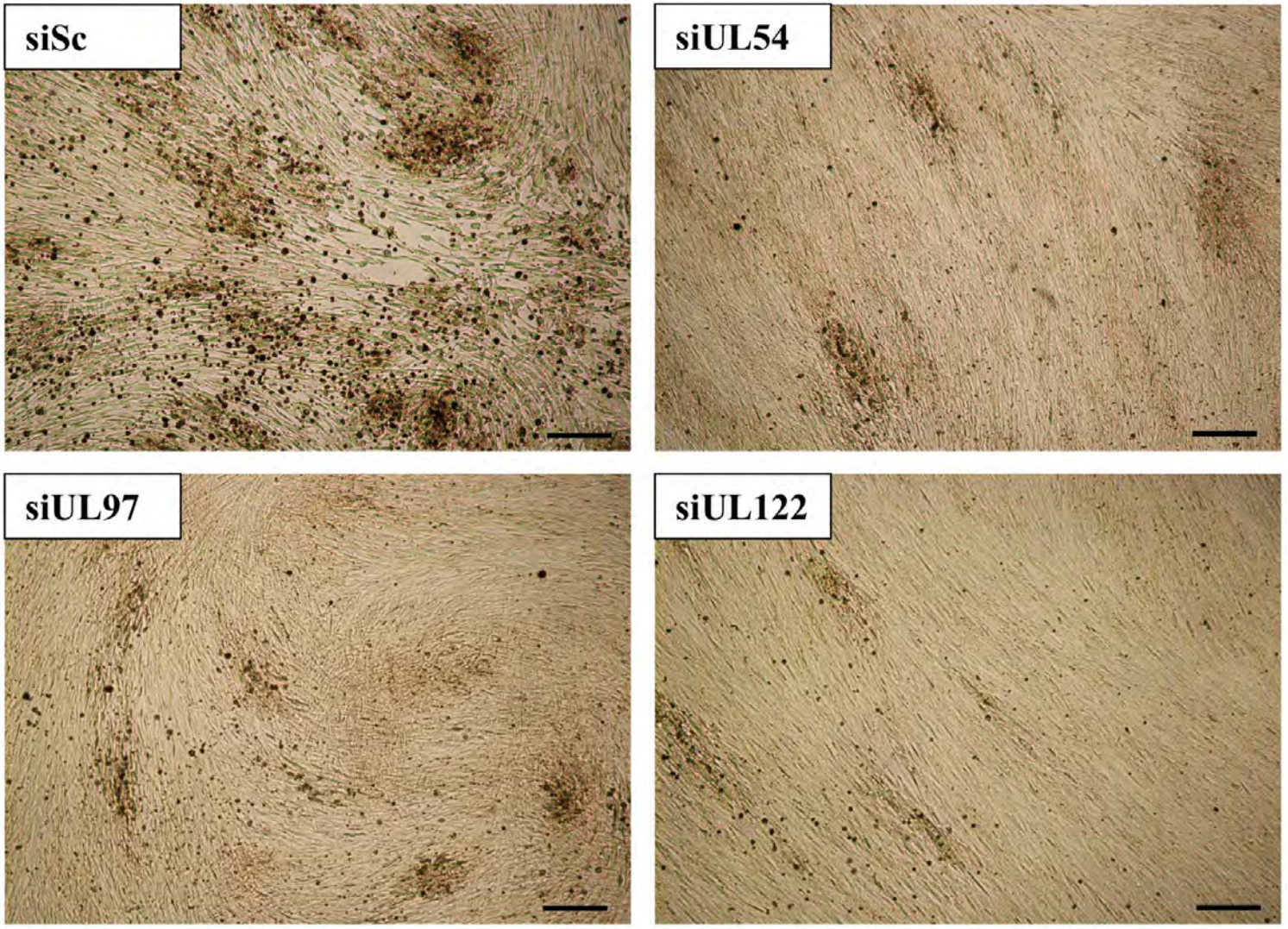
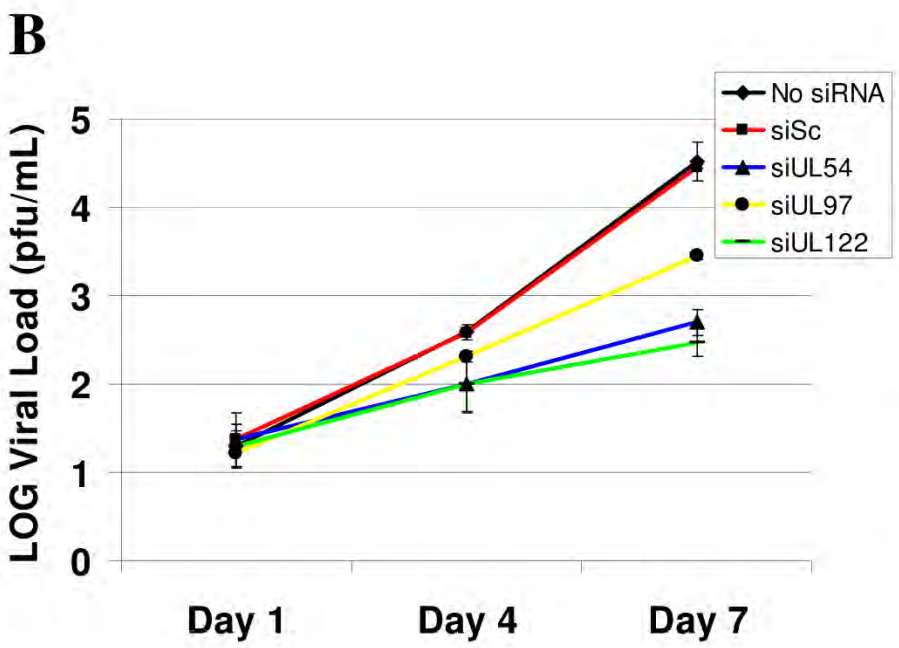
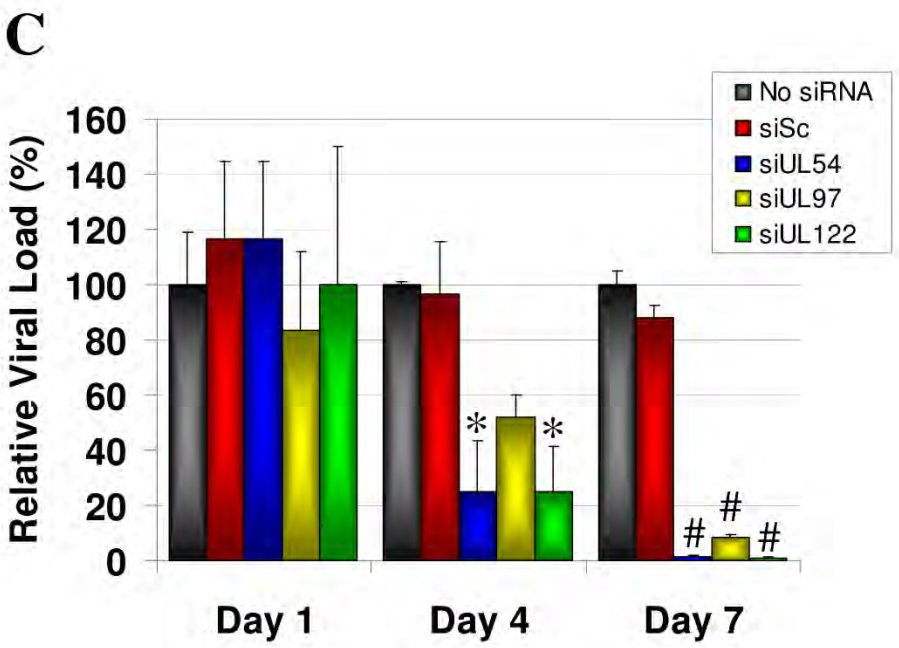
CMV siRNA Inhibition of CPE



siRNA Inhibition of CMV Progeny Production During Multiple Rounds of Replication (0.001 pfu/cell)





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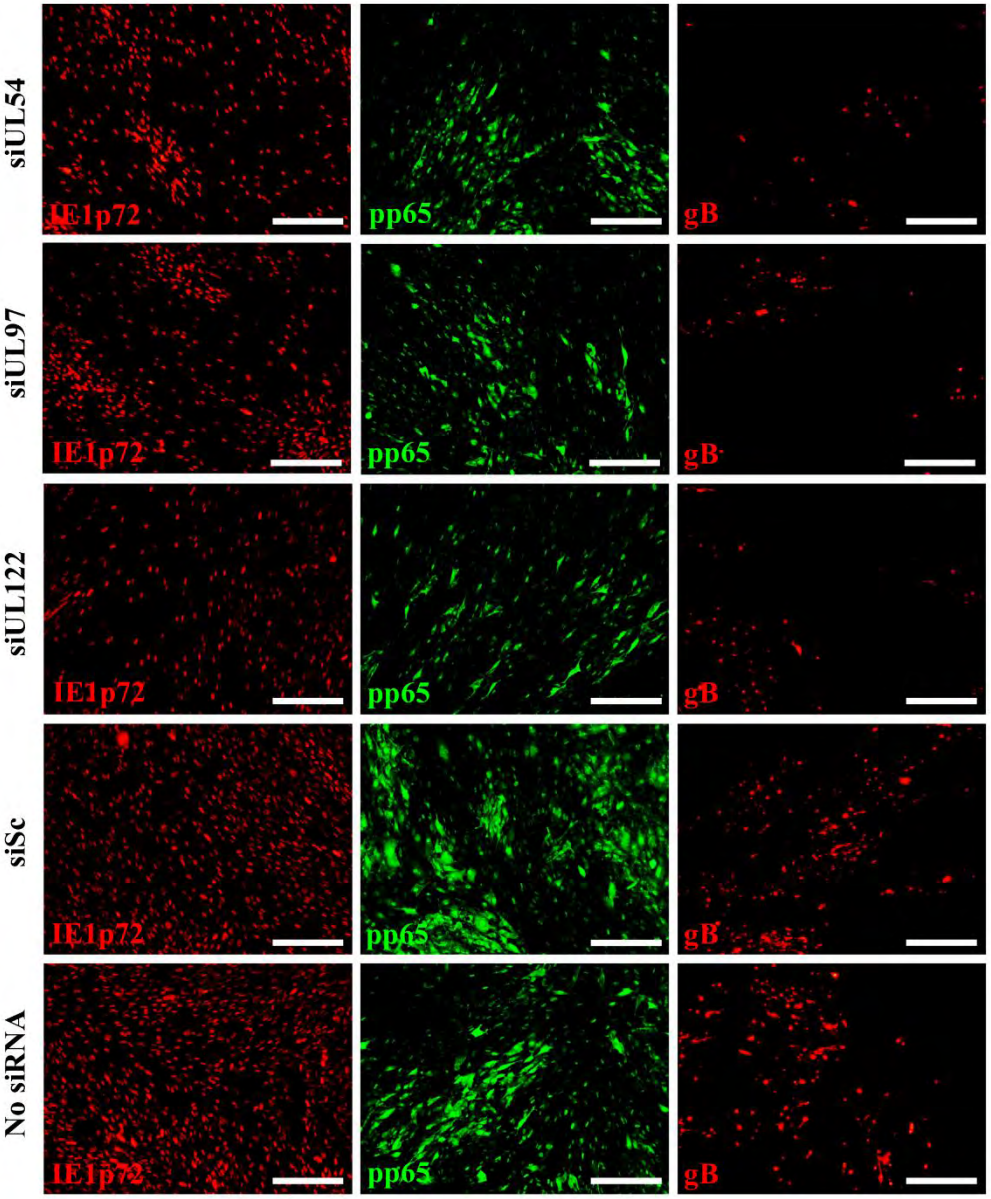

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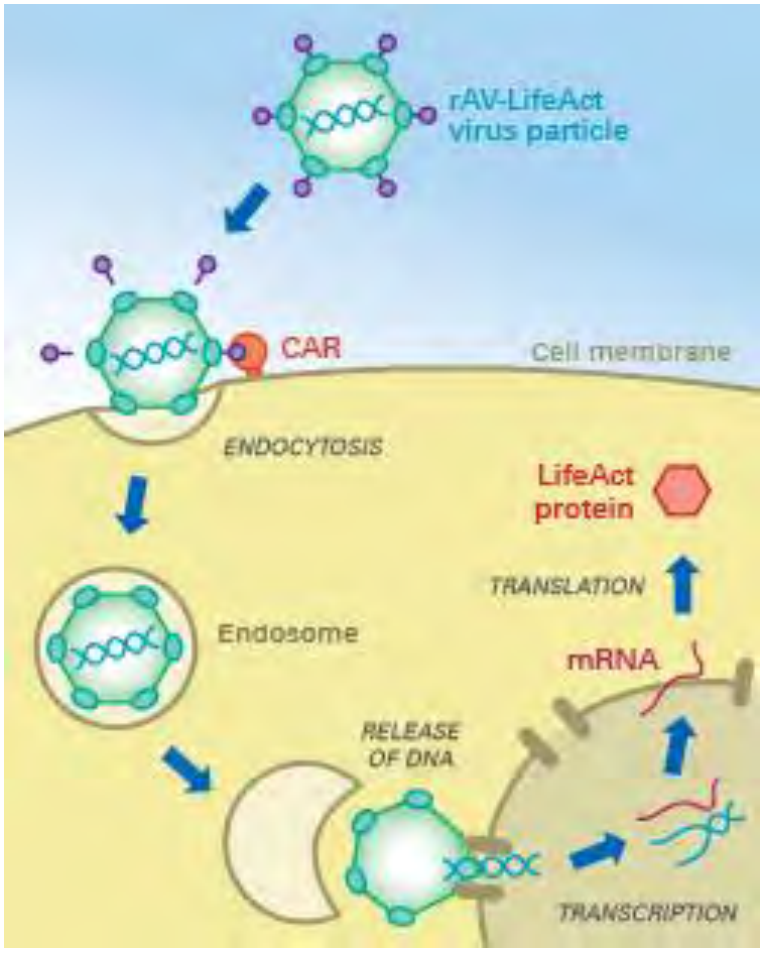
siRNA Delivery (Free siRNA)

- The major obstacle remaining for the development of successful siRNA therapeutics is optimization of the multiple components of an efficient delivery system.
- Naked RNA's are rapidly degraded by nucleases in serum
- Lack of targeted specificity results in rapid secretion by kidneys so large doses would be required for systemic administration
- RNA activates the innate immune response resulting in excessive cytokine release and inflammatory syndromes
- Poor transfection efficiencies into target cells

siRNA Delivery (Viral vectors)



Advantages

- Viral vectors have high gene transfection efficiency

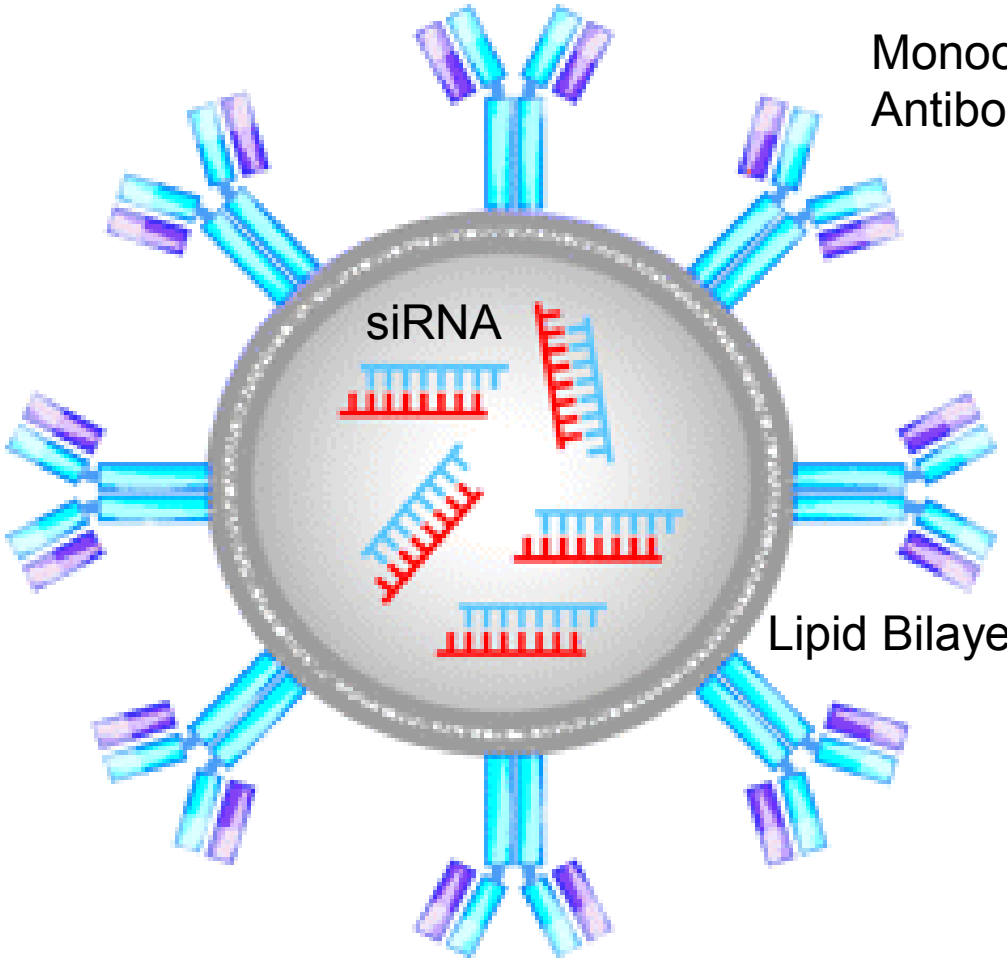
- Targeted delivery of siRNAs to cells

Disadvantages

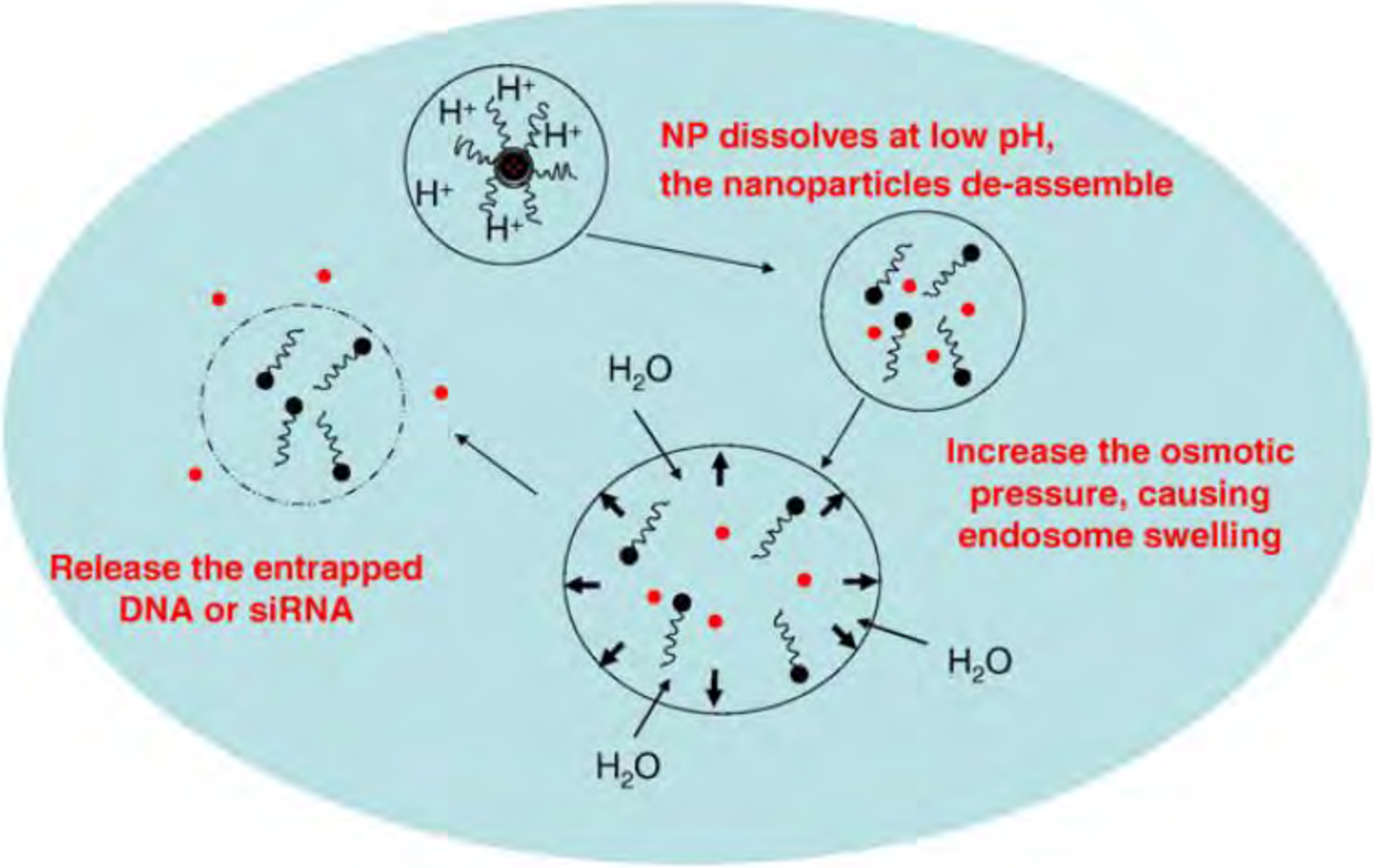
- Residual viral elements can cause insertional mutagenesis

- Viral vectors can also cause immunological problems

siRNA Delivery (Targeted nanoparticles)

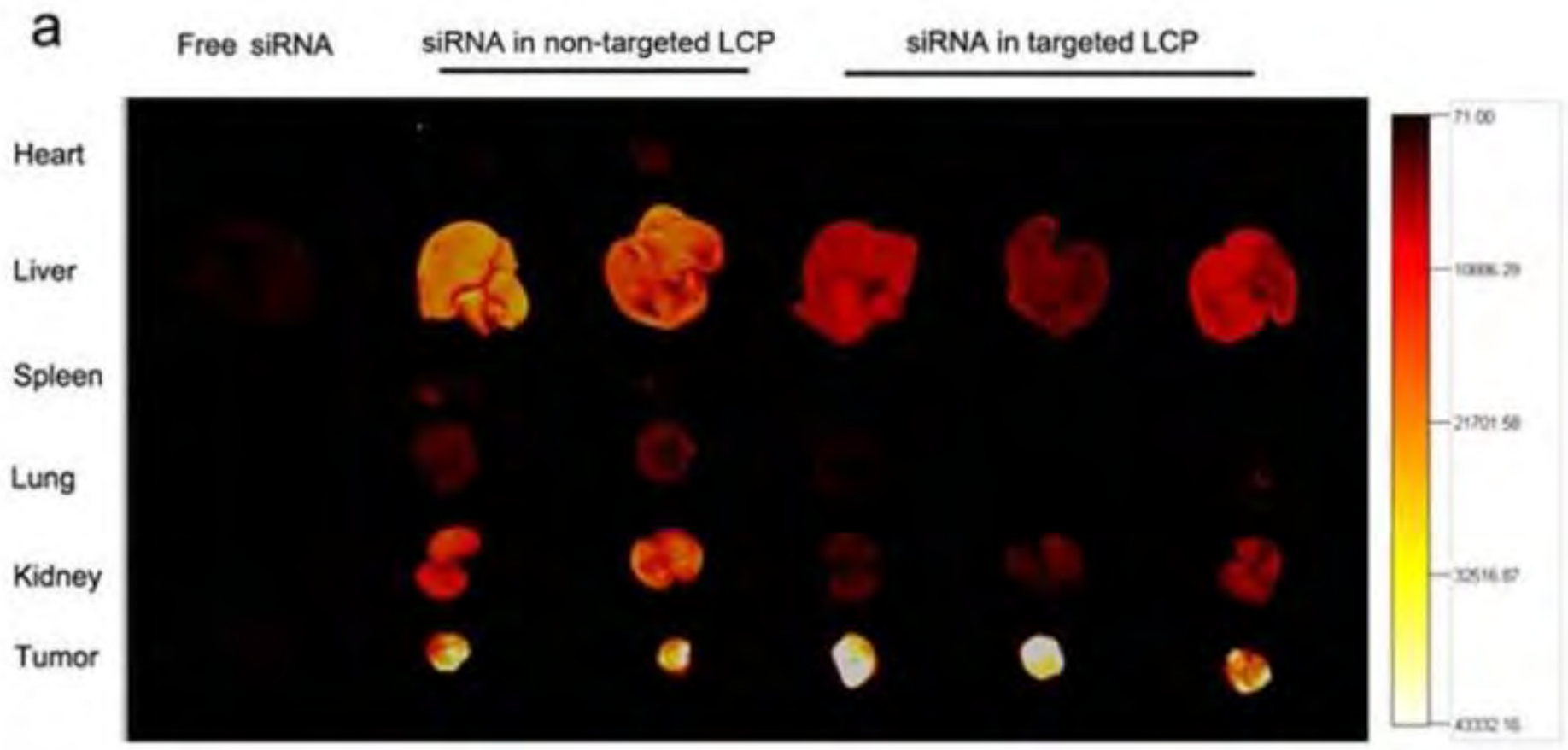


siRNA Delivery (Targeted nanoparticles)



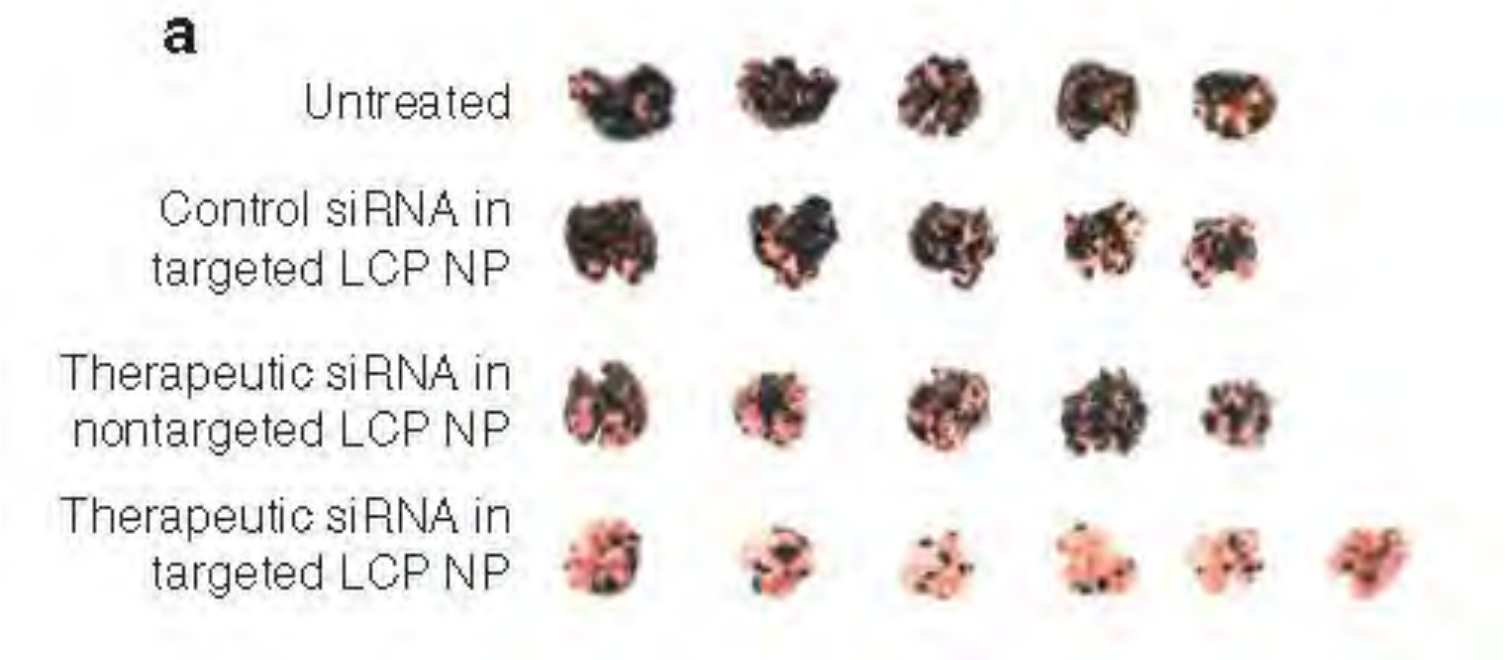
Li *et al.* 2011 J Controlled Release

siRNA Delivery (Targeted nanoparticles)



Yang *et al.* 2012 J Molecular Pharm

siRNA Delivery (Targeted nanoparticles)



Yang *et al.* 2012 J Molecular Pharm

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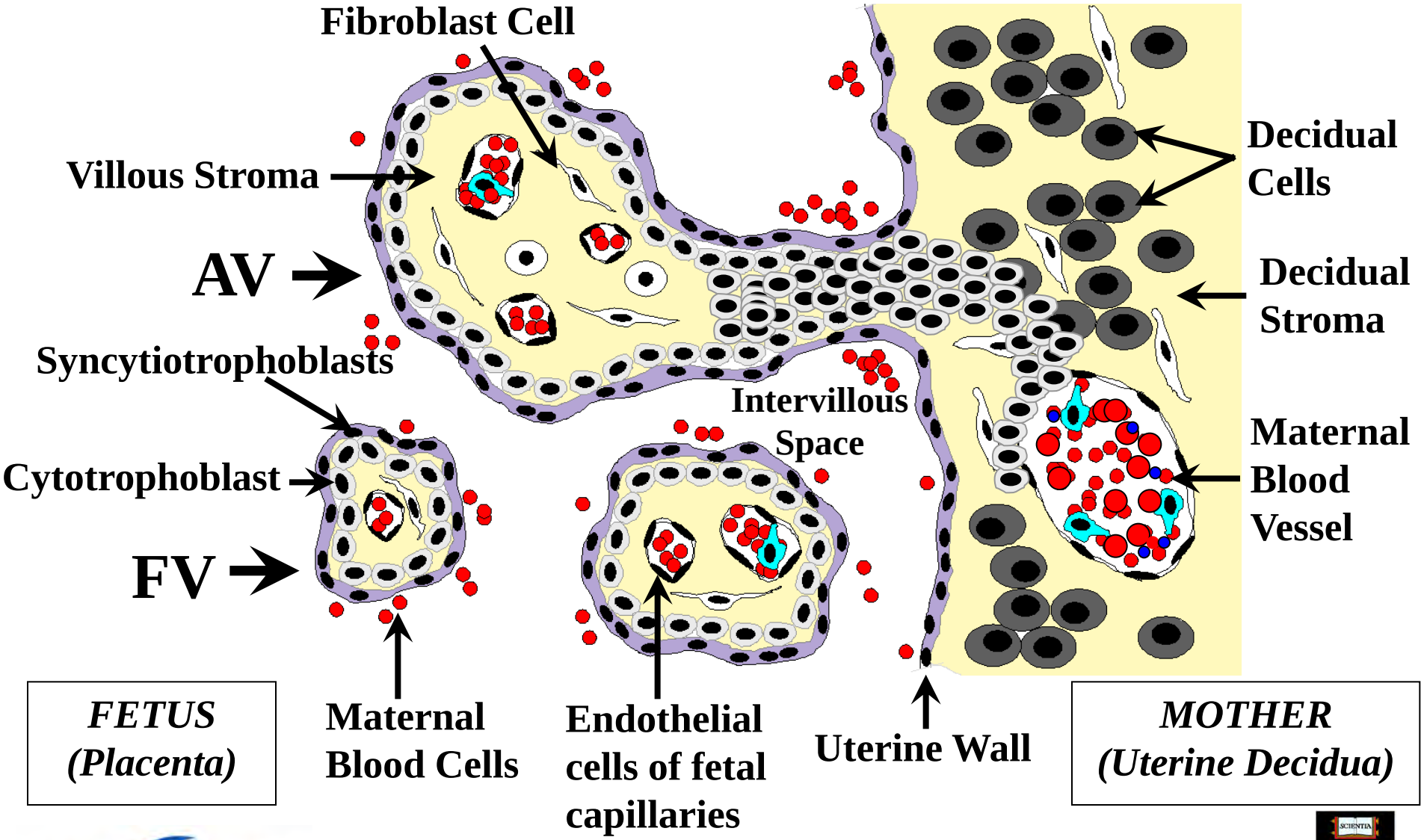
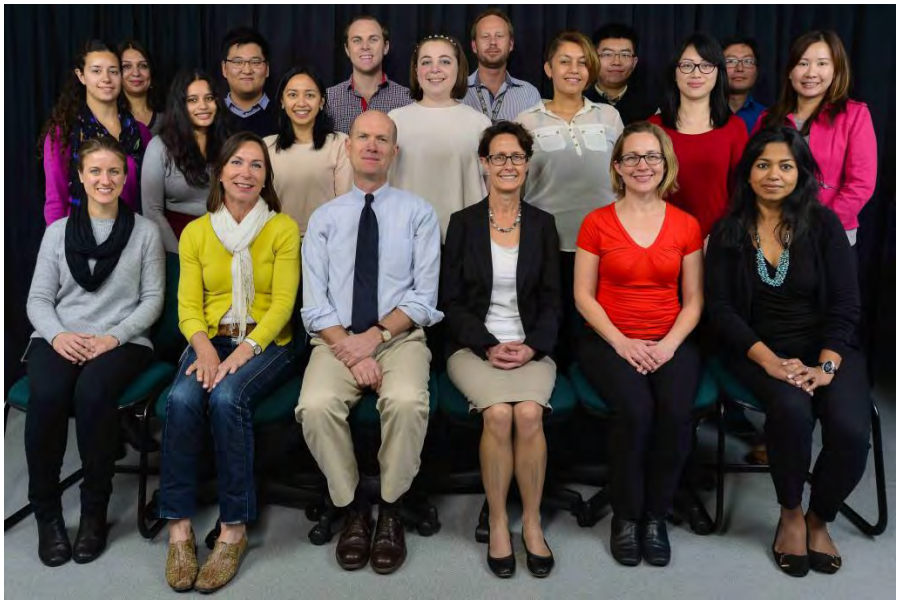


Summary

- siRNAs target mRNA gene transcripts and block specific gene expression
- siRNAs can be directed against specific viral transcripts to prevent viral replication and dissemination
- Specific nanoparticle delivery vehicles for targeted delivery of siRNAs have been developed and show efficacy and proof of principle
- More work is needed on the development of viral specific siRNA nanoparticles but show promise as a viable therapeutic option for the prevention and treatment of viral disease

Acknowledgments



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